

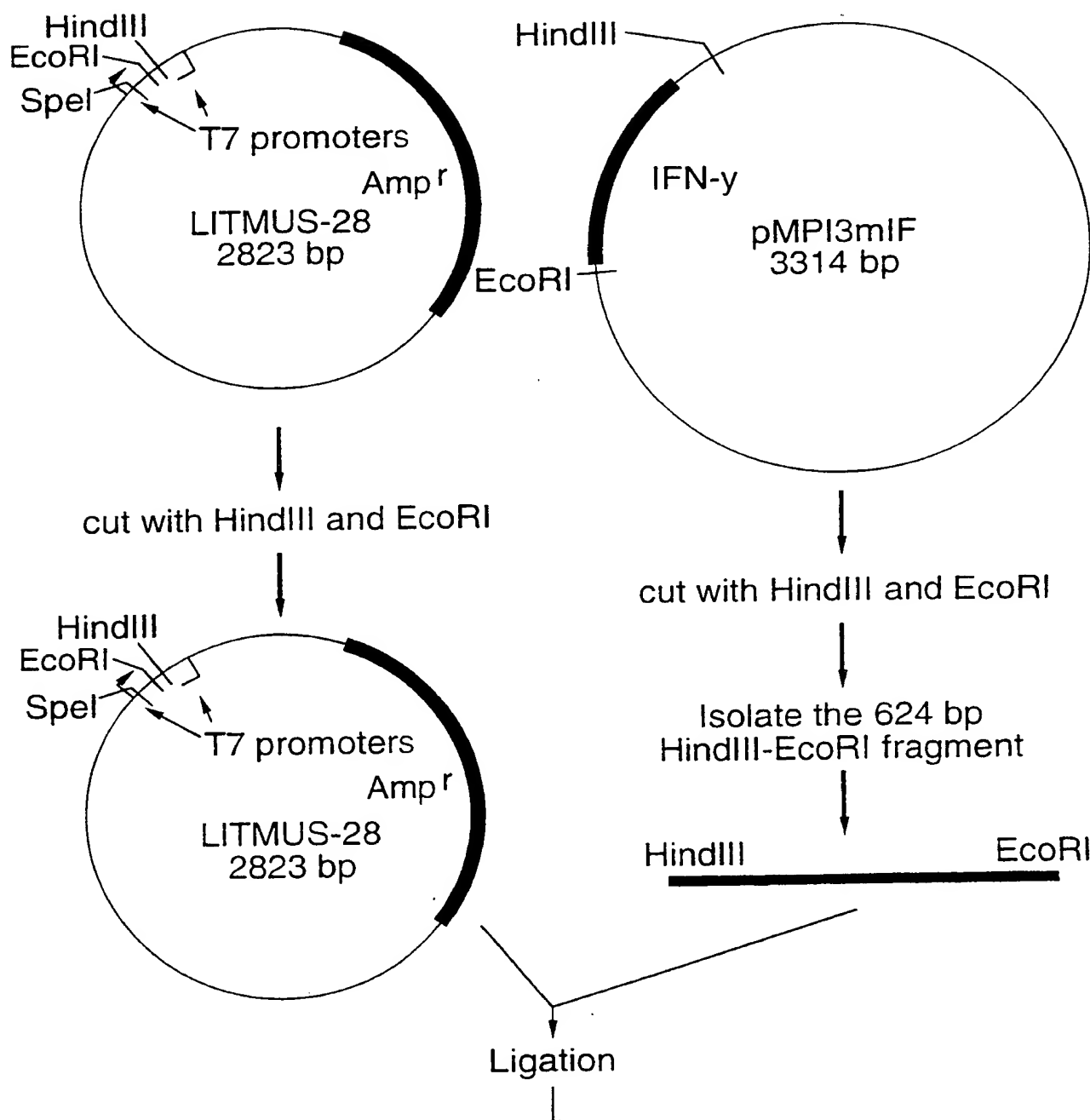
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Construction of pLITMUS-IFN- γ 

FIG.1A

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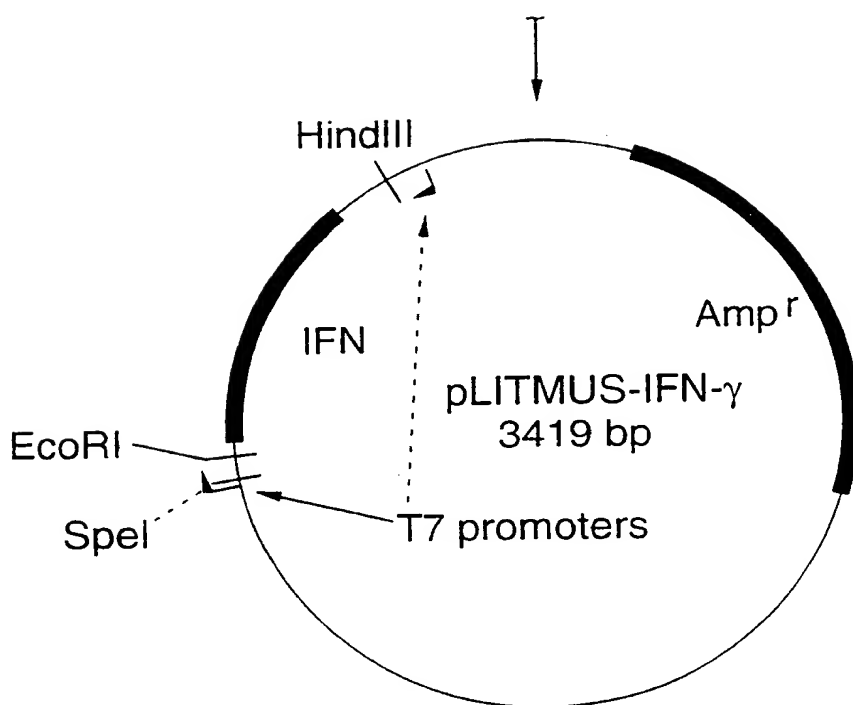


FIG.1B

FIG.2A

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Sequence of pLITMUS-IFN- γ

GTAACTACG TCAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTGTTTTATT
TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA
ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTGCGCC TTATTCCTTT
TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA ACGCTGGTGA AAGTAAAAGA
TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA
GATCCTTGAG AGTTTTCGCC CCGAAGAACG TTCTCCAATG ATGAGCACTT TTAAAGTTCT
GCTATGTGGC GCGGTATTAT CCGTGTGTA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT
ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA
TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC ATGAGTGATA ACGTGGCGC
CAACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT
GGGGGATCAT GTAACGCGC TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA
CGACGAGCGT GACACCACGA TGCTGTAGC AATGGCAACA ACGTTGCGCA AACTATTAAAC
TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA
AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC TGGTTTTATT CTGATAAATC
TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC
CTCCCGTATC GTAGTTATCT ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG
ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA
CTCATATATA CTTTAGATTG ATTTACCCCG GTTGATAATC AGAAAAGCCC CAAAAACAGG
AAGATTGTAT AAGCAAATAT TTAAATTGTA AACGTTAATA TTTTGTTAAA ATTTCGGTTA
AATTTTTGTT AAATCAGCTC ATTTTTTAAC CAATAGGCCG AAATCGGCAA AATCCCTTAT
AAATCAAAAG AATAGCCCGA GATAGGGTGT AGTGTGTGTT CAGTTTGGAA CAAGAGTCCA
CTATTAAAGA ACGTGGACTC CAACGTCAAA GGGCGAAAAA CCGTCTATCA GGGCGATGGC
CCACTACGTG AACCATCACC CAAATCAAGT TTTTTGGGGT CGAGGTGCCG TAAAGCACTA
AATCGGAACC CTAAAGGGAG CCCCCGATTT AGAGCTTGAC GGGGAAAGCG AACGTGGCGA
GAAAGGAAGG GAAGAAAGCG AAAGGAGCGG GCGCTAGGGC GCTGGCAAGT GTAGCGGTCA
CGCTGCGCGT AACCACCACA CCGCGCGCGC TTAATGCGCC GCTACAGGGC GCGTAAAAGG
ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA TCCCTTAACG TGAGTTTTTCG
TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT CTTCTTGAGA TCCTTTTTTTT
CTGCGCGTAA TCTGCTGCTT GCAAACAAAA AAACCACCGC TACCAGCGGT GGTTTGTGTTG
CCGGATCAAG AGCTACCAAC TCTTTTTTCCG AAGGTAAGT GCTTCAGCAG AGCGCAGATA
CCAAATACTG TTCTTCTAGT GTAGCCGTAG TTAGGCCACC ACTTCAAGAA CTCTGTAGCA
CCGCCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG CTGCTGCCAG TGGCGATAAG
TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG ATAAGGCGCA GCGGTGCGGC
TGAACGGGGG GTTCGTGCAC ACAGCCCAGC TTGGAGCGAA CGACCTACAC CGAACTGAGA
TACCTACAGC GTGAGCTATG AGAAAGCGCC ACGCTTCCCG AAGGGAGAAA GGCGGACAGG
TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC AGGGGGAAAC

FIG.2B

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GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT GACTTGAGCG TCGATTTTGT
TGATGCTCGT CAGGGGGGCG GAGCCTATGG AAAAACGCCA GCAACGCGGC CTTTTTACGG
TTCCTGGCCT TTTGCTGGCC TTTTGCTCAC ATGTAATGTG AGTTAGCTCA CTCATTAGGC
ACCCCAGGCT TTACACTTTA TGCTTCCGGC TCGTATGTTG TGTGGAATTG TGAGCGGATA
ACAATTTTAC ACAGGAAACA GCTATGACCA TGATTACGCC AAGCTACGTA ATACGACTCA
CTAGTGGGCA GATCTTCGAA TGCATCGCGC GCACCGTACG TCTCGAGGAA TTCCCGGGGA
TCCCTGCAGT TATTGGGACA ATCTCTTCCC CACCCCGAAT CAGCAGCGAC TCCTTTTCCG
CTTCTGAGG CTGGATTCCG GCAACAGCTG GTGGACCACT CGGATGAGCT CATTGAATGC
TTGGCGCTGG ACCTGTGGGT TGTTGACCTC AAACCTTGCA ATACTCATGA ATGCATCCTT
TTTCGCCCTG CTGTTGCTGA AGAAGGTAGT AATCAGGTGT GATTCAATGA CGCTTATGTT
GTTGCTGATG GCCTGATTGT CTTTCAAGAC TTCAAAGAGT CTGAGGTAGA AAGAGATAAT
CTGGCTCTGC AGGATTTTCA TGTCACCATC CTTTITGCCAG TTCCTCCAGA TATCCAAGAA
GAGACTCTTT TCTTCCACAT CTATGCCACT TGAGTTAAAA TAGTTATTCA GACTTCTAG
GCTTTCAATG ACTGTGCCGT GGCAGTAACA GCCAGAAACA GCCATGAGGA AGAGCTGCAA
AGCCAAGATG CAGTGTGTAG CGTTCATGAT TAGATTAAAC TAAATAATTG TACTTTGTAA
TATAATGATA TATATTTTCA CTTTATCTCA TTTGAGAATA AAAATGTTTT TGTTTAACCA
CTGCATGATG TAAGCTTCCC ATGGTGACGT CACCGGTTCT AGATACCTAG GTGAGCTCTG
GTACCCCTCTA GTCAAGGCCT TAAGTGAGTC GTATTACGGA CTGGCCGTCG TTTTACAACG
TCGTGACTGG GAAAACCTG GCGTTACCCA ACTTAATCGC CTTGCAGCAC ATCCCCCTTT
CGCCAGCTGG CGTAATAGCG AAGAGGCCCG CACCGATCGC CCTTCCCAAC AGTTGCGCAG
CCTGAATGGC GAATGGCGCT TCGCTTGGTA ATAAAGCCCG CTTCGGCGGG CTTTTTTTTT

Utilized T7 promoter 3199 - 3216

Transcription start site 3198

IFN-(specific sequence 2530 - 3027

Transcript sequence 2464 - 3198

SpeI site used for linearization 2461

FIG.3A

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Sequence of the IFN- γ RNA Standard

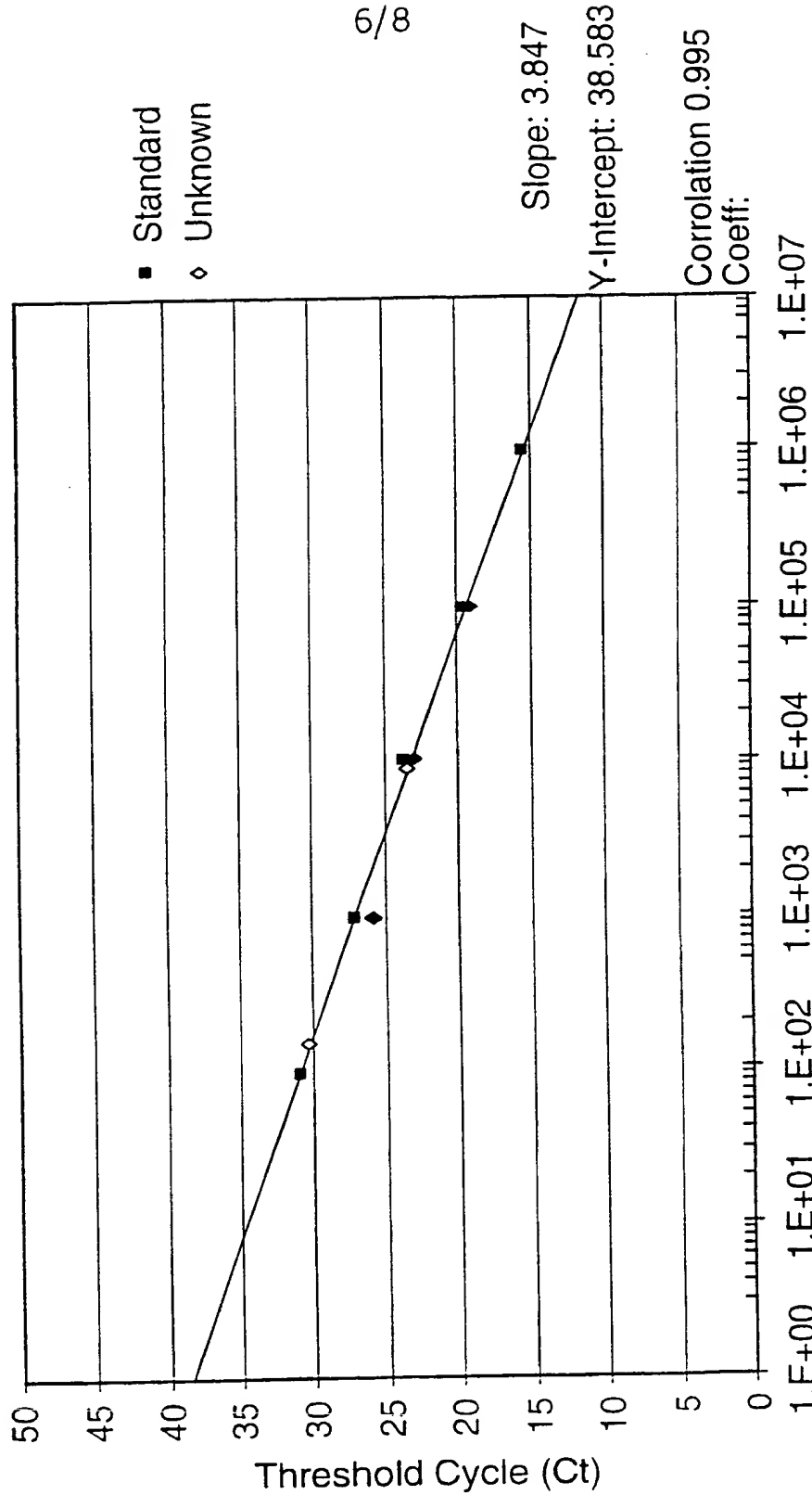
1 GCCUUGACUA GAGGGUACCA GAGCUCACCU AGGUAUCUAG AACCGGUGAC 50
51 GUCACCAUGG GAAGCUUACA UCAUGCAGUG GUUAAACAAA AACAUUUUUA 100
101 UUCUCAAUG AGAUAAAGUG AAAAUUAUAU UCAUUAUAU ACAAAGUACA 150
151 AUUAUUUAGG UAAUCUAAU CAUGAACGCU ACACACUGCA UCUUGGCUUU 200
201 GCAGCUCUUC CUCAUGGCUG UUUCUGGCUG UUACUGCCAC GGCACAGUCA 250
251 UUGAAAGCCU AGAAAGUCUG AAUAACUAU UUAACUCAAG UGGCAUAGAU 300
301 GUGGAAGAAA AGAGUCUCUU CUUGGAUAUC UGGAGGAACU GGCAAAAGGA 350
351 UGGUGACAUG AAAAUCCUGC AGAGCCAGAU UAUCUCUUUC UACCUCAGAC 400
401 UCUUUGAAGU CUUGAAAGAC AAUCAGGCCA UCAGCAACAA CAUAAGCGUC 450
451 AUUGAAUCAC ACCUGAUUAC UACCUUCUUC AGCAACAGCA AGGCGAAAAA 500
501 GGAUGCAUUC AUGAGUAUUG CCAAGUUUGA GGUCAACAAC CCACAGGUCC 550
551 AGCGCCAAGC AUUCA AUGAG CUCAUCCGAG UGGUCCACCA GCUGUUGCCG 600
601 GAAUCCAGCC UCAGGAAGCG GAAAAGGAGU CGCUGCUGAU UCGGGGUGGG 650
651 GAAGAGAUUG UCCCAAUAAC UGCAGGGAUC CCCGGGAAUU CCUCGAGACG 700
701 UACGGUGCGC GCGAUGCAUU CGAAGAUUC CCCACUAG 738

IFN- γ specific sequence 172 - 669

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Standard Curve: JC RT-PCR 92 γ -IFN



Starting Quantity

FIG.4

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RT-PCR Cycle Optimization for Lialuid Hybridization

PCR Cycle Optimization for IL5 Amplification

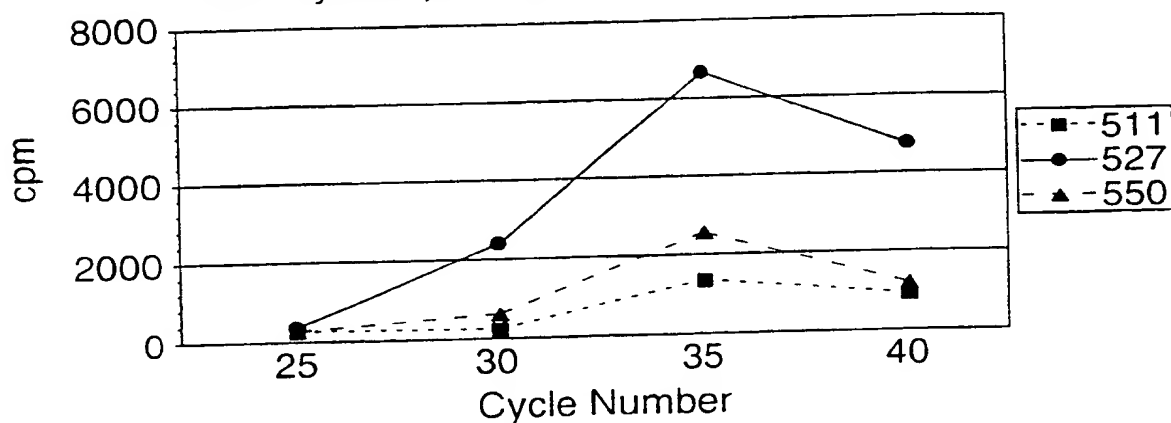


FIG.5A

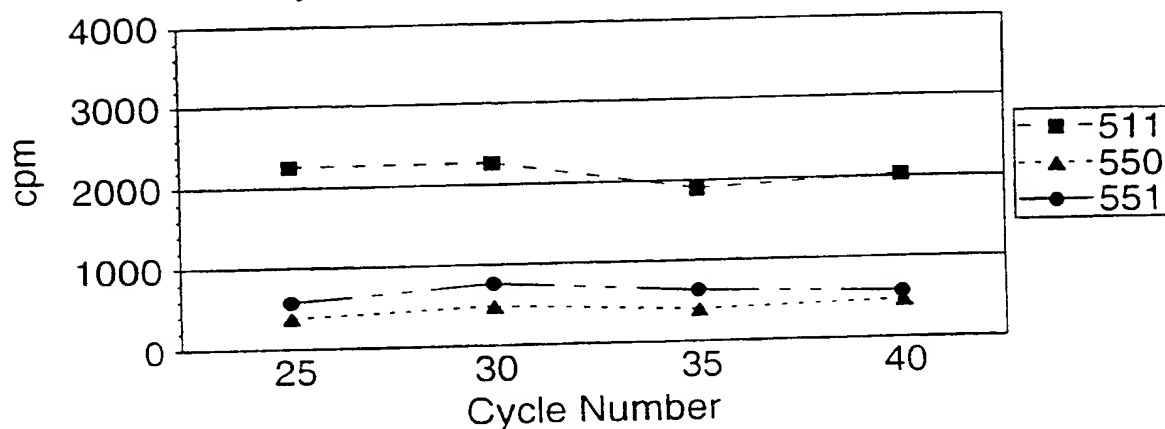
PCR Cycle Optimization for γ -IFN Amplification

FIG.5B

PCR Cycle Optimization for IL4 Amplification

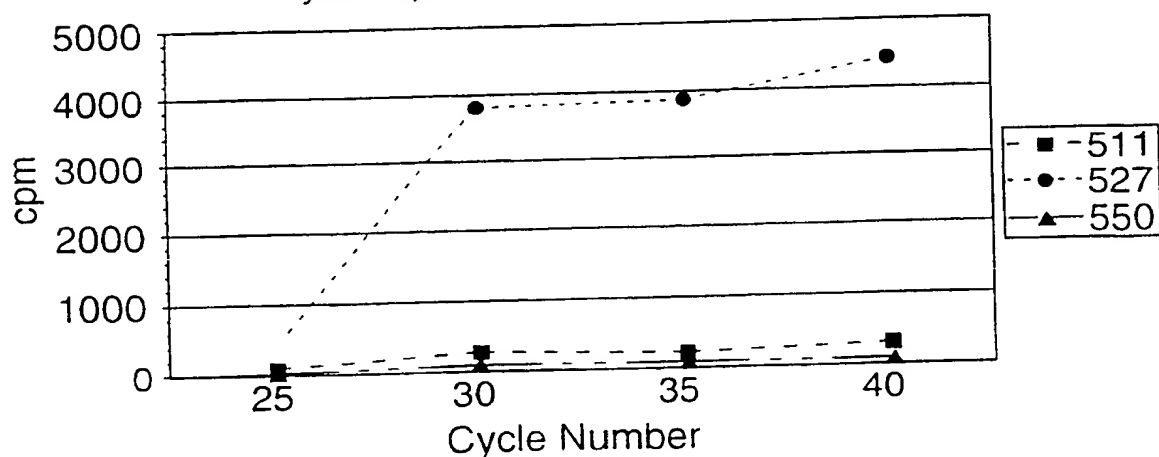


FIG.5C

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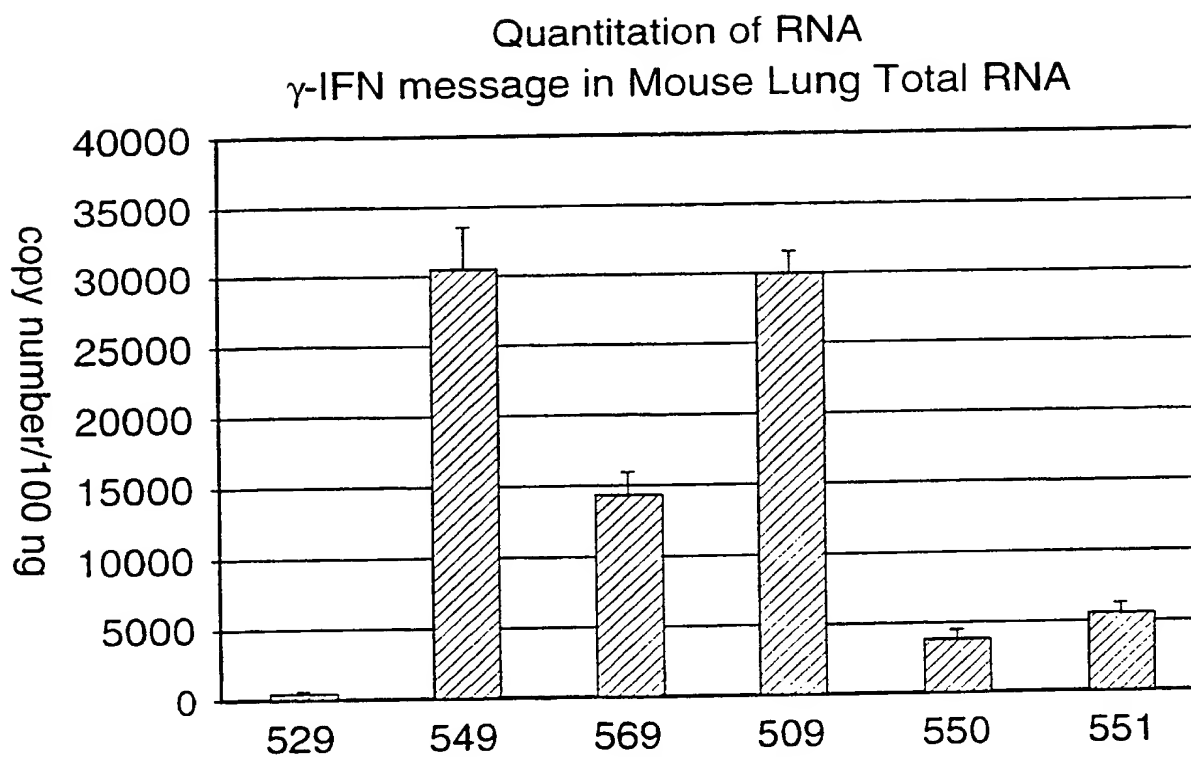


FIG.6